EPA Reviewer: Lisa Austin, Ph.D. **Signature:**
Registration Action Branch 1, Health Effects Division (7509C) **Date:** 7/30/09
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HED Executive Summary Cover for the attached OECD Formatted DATA EVALUATION RECORD

STUDY TYPE: 28-Day Oral Toxicity feeding-[mice]; OPPTS 870.3100 [§82-1a] (rodent); OECD 408.

PC CODE: 118203 **DP BARCODE:** D349929

TEST MATERIAL (PURITY): BAS 800 H (94.2%)

SYNONYMS: AC 433379; BASF Reg. No. 4054449, saflufenacil

CITATION: Kaspers, U., Strauss, V., Kaufmann, W. et. al. (2007) BAS 800 H – Range Finding Study in C57BL/6NCrl mice- Administration in the diet for 4 weeks. Experimental toxiciology and Ecology BASF Aktiengesellschaft, Ludwigshafen, Germany. Laboratory report number 31S0414/01148, May 11, 2007. MRID 47128110. Unpublished.

SPONSOR: BASF Aktiengesellschaft

EXECUTIVE SUMMARY:

In a 28-day oral toxicity study (MRID 47128110*) BAS 800 H (94.2% a.i., Lot # COD 000227)* was administered to C57BL/6NCrl mice, 5/sex/dose in the diet at dose levels of 0, 50, 150, 450, 1350, or 4050 ppm (equivalent to M\F: 0, 12.8/17.9, 36.6/63.4, 112/153, 335/446, 882/1630 mg/kg bw/d).

There were no treatment related effects on mortality, clinical signs or gross pathology. Bodyweight (5.5-14.4%) and bodyweight change (38.3-69.6%) were significantly decreased in males at doses >1350 ppm. A decrease in food consumption (NSS) was observed in high dose males only (9-23%). In females, these parameters were comparable to controls.

In males, red blood cells were significantly decreased (10-20%) at 150, 1350, and 4050 ppm. Hemoglobin (Hb, 8-26%) and hematocrit (Hct, 8-29%) were significantly decreased at doses >150 ppm. Mean corpuscular volume (MCV, 3-11%) and mean corpuscular hemoglobin (MCH, 5-9%) were significantly decreased at doses >450 ppm. Examination of RBC morphology in males revealed increased anisocytosis, microcytosis, and polychromasia at 4050 ppm. Increased polychromasia was also seen in the erythrocytes of the males at 1350 ppm. In females, Hb, MCH, and MCHC were significantly decreased at doses >1350 ppm. Hct and MCV were also decreased at 4050 ppm. Increased anisocytosis and polychromasia were measured in erythrocytes of females at 4050 ppm. These hematological changes were indicative of a sideroblastic anemia.

Alanine (ALT, 308-908%) and aspartate aminotransferase (AST, 46-168%) activities in males were dose-dependent and significantly elevated at 150, 450, 1350, and 4050 ppm. Significantly higher alkaline phosphatase (AP, 95%) activities were also seen in males at 4050 ppm. Statistically significant increase in urea(CHECK BUN) (25-37%) and total bilirubin (25-45%) levels were found in males at ≥150 ppm. In females at 1350 and 4050 ppm AP (24-29%) and ALT (92-153%) activities were significantly elevated.

In males, there were statistically significant increases in absolute (11-22%) and relative (18-44%) liver weights at ≥150 ppm and increase in spleen weight at 4050 ppm. In females, the only treatment-related findings on organ weights were statistically significant increase of absolute (35-40%) and relative (39-41%) liver weights at 1350 and 4050 ppm. All other statistically significant weight changes (kidneys, thymus, and brain) were either secondary to the significant terminal body weight decrease in the high dose group or are of no biological significance due to lack of related histopathological findings.

In the liver, changes that increased in severity and/or incidence included centrilobular fatty changes (♂ ≥150 ppm, 5/5 vs 0/5 controls; ♀ ≥450 ppm, 5/5 vs 3/5 controls), minimal lymphoid infiltration (♂ ≥150 ppm, 4-5/5 vs 0/5 controls; ♀ ≥1350 ppm, 5/5 vs 0/5 controls), and extramedullary hematopoiesis (♂ ≥450 ppm, 4-5/5 vs 0/5 controls). In the spleen, the only finding was extramedullary hematopoiesis which increased in incidence and severity (♂ ≥1350 ppm, 5/5 vs 2/5 controls; ♀ 4050 ppm, 5/5 vs 0/5 controls). A slight increase of apoptotic necrosis of lymphocytes was observed in the thymus of treated males at ≥150 (2-3/5 vs 1/5 controls).

The LOAEL in males is 150 ppm (36.6 mg/kg bw/d) based on hematological and clinical chemistry effects (increased alanine aminotransferase, aspartate aminotransferase, urea and total bilirubin as well as decreased hemoglobin and hematocrit), and liver pathology (increased weight, lymphoid infiltration and centrilobular fatty change). The NOAEL is 50 ppm (12.8 mg/kg bw/d). The LOAEL in females is 450 ppm (153.1 mg/kg bw/d) based on moderate centrilobular fatty change in the liver. The NOAEL is 150 ppm (63.4 mg/kg bw/d).

This 28-day oral toxicity study in mice is acceptable, non-guideline range-finding study and does not satisfy the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in mice.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Flagging and Data Confidentiality statements were provided.

Reviewer #: Steve Wong, Ph.D. , Date April 16, 2008

APPLICANT: BASF Corporation

STUDY TYPE: Short-Term Oral (28-day) Toxicity feeding study in mice; OECD 407.

TEST MATERIAL (PURITY): BAS 800 H (94.2%)

SYNONYMS: AC 433379; BASF Reg. No. 4054449

CITATION: Kaspers, U., Strauss, V., Kaufmann, W. et al. (2007) BAS 800 H Range finding study in C57BL/6NCrl mice; Administration in the diet over 4 weeks. Experimental Toxicology and Ecology, BASF Aktiengesellschaft 67056 Ludwigshafen, FGR. Report Number(s) 31S0414/01148. BASF Doc ID 2005/1011556. May 11, 2007. Unpublished. [PMRA # 1546975]

SPONSOR: BASF Corporation, Agricultural Products, 26 Davis Drive, Research Triangle Park, NC 27709

EXECUTIVE SUMMARY

In a 28-day toxicity study, BAS 800 H (94.2%) was administered daily in the diet to C57BL/6NCrl mice, 5/sex/dose, at 0, 50, 150, 450, 1350 and 4050 ppm (♂ = 0, 12.8, 36.6, 112, 335, 882; ♀ = 0, 17.9, 63.4, 153, 446, 1621 mg/kg bw/d, respectively). Systemic toxicity was observed in males at ≥150 ppm and in females at ≥450 ppm. Systemic toxicity included impaired body weight and body-weight gain, changes in several hematological parameters indicative of a sideroblastic anemia, and pathology of the liver and spleen. The red blood cells, the liver and spleen were recognized as targets of BAS 800 H. The LOAEL established in males was 150 ppm (36.6 mg/kg bw/d) based on hematological and clinical chemistry effects (increased alanine aminotransferase, aspartate aminotransferase, urea and total bilirubin as well as decreased hemoglobin and hematocrit), and liver pathology (increased weight and centrilobular fatty change). The NOAEL in males was 50 ppm (12.8 mg/kg bw/d). The LOAEL established in females was 450 ppm (153.1 mg/kg bw/d) based on moderate centrilobular fatty change in the liver. The NOAEL in females was 150 ppm (63.4 mg/kg bw/d).

COMPLIANCE: Signed and dated GLP, Quality Assurance, Flagging, and Data Confidentiality statements were provided.

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I. MATERIALS AND METHODS

A. MATERIALS:

1.

Test material:	BAS 800 H
Description:	Solid-crystalline / light-beige; stored at room temperature
Lot/Batch #:	COD - 000227
Purity:	94.2% a.i.
Compound stability:	The stability under the storage conditions present in this study was guaranteed by the Certificate of Analysis. The homogeneity of the test material was confirmed by analysis.
CAS #:	372137-35-4

2.

Vehicle and/or positive control: BAS 800 H was administered in the diet.

3. **Test animals:**

Species:	Mouse	
Strain:	C57BL/6NCrl	
Age/weight at study initiation:	Age: 40±1 day ; Mean weight: ♂ = 23.4 , ♀ = 18.3 g	
Source:	Charles River, Germany	
Housing:	Singly in polycarbonate cages, type M1 (floor area about 200 cm^2) with wire cover.	
Diet:	Kliba maintenance diet mouse/rat "GLP", meal, supplied by Provimi Kliba SA, Kaiseraugst, Switzerland. *ad libitum*	
Water:	Tap water *ad libitum*	
Environmental conditions:	**Temperature:** **Humidity:** **Air changes:** **Photoperiod:**	20-24°C 30-70% 10/h 12h dark / 12h light
Acclimation period:	At least five days prior to application	

B. STUDY DESIGN:

1. **In life dates** Start: July 21, 2004 End: August 23, 2004

2. **Animal assignment**: Animals were assigned to test groups via a randomization protocol provided by a computer. The test groups are noted in Table 1.

Table 1: Study design

	♂						♀					
ppm	0	50	150	450	1350	4050	0	50	150	450	1350	4050
mg/kg bw/d	0	12.8	36.6	112	335	882	0	17.9	63.4	153	446	1621
N	5	5	5	5	5	5	5	5	5	5	5	5

3. Diet preparation and analysis

For each concentration, BAS 800 H was weighed out and mixed with a small amount of food. Then appropriate amounts of food, depending on the dose group, were added to this premix to obtain the desired concentrations. The BAS 800 H preparations were mixed once before the study. The stability of BAS 800 H in the diet was proven with a comparable batch of BAS 800 H for a period of up to 49 days at room temperatures. Before the start of the administration period, three samples (from the top, middle, and bottom of the container) were removed from the lowest and highest concentrations to verify the concentration and homogeneity of BAS 800 H. A single sample was removed from the intermediate doses for concentration verification.

Results

Homogeneity analysis: For the 50 ppm preparation, the mean concentration was 100.0±1.5% of the nominal concentration. For the 4050 ppm preparation, the mean concentration was 101.7±0.2% of the nominal concentration.

Stability analysis: In feed, the material was stable for a period of 49 days (0 D: 100.0% of nominal; 9 D: 102.7% of nominal; 34 D: 95.3% of nominal; 49 D: 97.9% of nominal).

Concentration analysis: Mean concentrations ranged from 98.2 to 102.8% of the nominal concentrations.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

4. Statistics

Parameter	Statistical Test*	References
Food consumption, body weight, body weight change, food efficiency	A comparison of each group with the control group using the Dunnett-test (two-sided) for the hypothesis of equal means	Dunnett, C.W. (1955): A multiple comparison procedure for comparing several treatments with a control. JASA, Vol. 50, 1096-1121 Dunnett, C.W. (1964). New tables for multiple comparisons with a control. Biometrics, Vol. 20, 482-491
Clinical pathology parameters, Except reticulocytes and differential blood count	Non-parametric one-way analysis using Kruskal-Wallis test (two-sided).If the resulting p-value was equal or less than 0.05, a pair-wise comparison of each dose group with the control group was performed using Wilcoxon-test (two-sided) for the equal medians	Siegel S. (1956): Non-parametric statistics for behavioral sciences. McGraw-Hill New York
Weight parameters	Non-parametric one-way analysis using Kruskal-Wallis- test (two-sided). If the resulting p-value was equal or less than 0.05, a pair-wise comparison of each dose group with the control group was performed using the Wilcoxon-test (two-sided) for the equal medians	Miller, , R. G. (1981): Simultaneous Statistical Inference Springer-Verlag New York Inc, 165-167. International Mathematical and Statistical Libraries, Inc., 2500 Park West Tower One, Houston, Texas 77042-3020, USA, nakl-1-nakl-3. Nijenhuis, A. and WILF H.S. (1978): Combinatorial Algorithms, Academic Press, New York, 32-33. Hettmannsperger, T. P. (1984): Statistical Inference based on Ranks, John Wiley & Sons New York, 132- 140.
* Significantly different (p <0.05) from the control. ** Significantly different (p <0.01) from the control		

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C. METHODS:

1. Observations:

The mice were examined for signs of toxicity and mortality twice a day on weekdays and once a day on weekends and public holidays. Detailed clinical observations were conducted for all mice prior to the dosing and thereafter at weekly intervals. Parameters examined were as follows:

Skin / fur	Respiration	Urine	Pupil size	Palpebral closure
Posture	Tremors	Lacrimation	Impairment of gait	Activity / arousal level
Salivation	Convulsions	Exophthalmus	Abnormal movements	Feces (appearance/consistency)
Abnormal behaviour during handling				

2. Body weight:

Body weight was determined before the start of the administration period in order to randomize the animals. The weights were then determined on day 0 and weekly thereafter.

3. Food consumption and compound intake:

Food consumption for each mouse was determined weekly over a 7-day period and mean daily diet consumption was calculated as g food/kg bw/d. Food efficiency (body weight gain in g/food consumption in g per unit time X 100) and compound intake (mg/kg bw/d) values were calculated as time-weighted averages from the consumption and body weight gain data.

4. Ophthalmoscopic examination: An ophthalmoscopic examination was not conducted.

5. Hematology & clinical chemistry:

At the end of the dosing period, blood was removed from fasted animals from the retro-orbital venous plexus or after decapitation. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (Hct)*	X	Leukocyte differential count*
X	Hemoglobin (Hb)*	X	Mean corpuscular Hb (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpuscular Hb concentration (MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)*
X	Blood clotting measurements*	X	Reticulocyte count
X	Platelet count		
X	Clotting parameters - Prothrombin time (Thromboplastin time, Clotting time)		

* Recommended for subchronic rodent studies based on OECD 407 and EPA Guideline 870.3100

b. Clinical chemistry

	ELECTROLYTES				**OTHER**
X	Calcium*	X	Chloride*	X	Albumin*
X	Phosphorus*	X	Magnesium		Blood creatinine*
X	Potassium*	X	Sodium*	X	Blood urea nitrogen*
	ENZYMES			X	Total Cholesterol
X	Alkaline phosphatase (AP)			X	Globulins

	Cholinesterase (ChE)	X	Glucose*
X	Creatine phosphokinase	X	Total bilirubin*
	Lactic acid dehydrogenase (LDH)	X	Total serum protein (TP)*
X	Serum alanine amino-transferase (ALT/ SGPT)*	X	Triglycerides
X	Serum aspartate amino-transferase (AST/ SGOT)*		Serum protein electrophores
X	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		
X	cyanide-insensitive palmitoyl-CoA-oxidation		

* Recommended for subchronic rodent studies based on OECD 407 and EPA Guideline 870.3100

6. Urinalysis* Urine parameters were not analyzed in this study. (* Not required)

7. Sacrifice and pathology

All mice that died and those sacrificed at study termination were subject to gross pathologic examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs were weighed.

Digestive system				**Cardiovacular/Hematologic**				**Neurologic system**	
	Tongue				Aorta	xx	Thymus	xx	Brain
	Salivary glands			xx	Heart*	x	Bone marrow	x	Peripheral nerve
xx	Liver*+			xx	Spleen*	x	Lymph nodes	x	Spinal cord (3 levels)
	Esophagus	x	Rectum	**Urogenital system**					Pituitary
x	Stomach	x	Jejunum	xx	Kidneys*+		Urinary bladder		Eyes (optic nerve)
x	Duodenum	x	Cecum	xx	Testes+	xx	Epididymides	**Glandular organs**	
x	Ileum	x	Colon	x	Prostate	x	Seminal vesicles	xx	Adrenal gland*+
x	Gall bladder			xx	Ovaries	xx	Uterus		Lacrimal gland
	Pancreas				Oviducts and vagina				Mammary gland
Respiratory system				**Other**					Parathyroid
x	Trachea			x	Bone	x	Skin	x	Thyroid
	Nose		Larynx		Skeletal muscle				
	Pharynx			x	All gross lesions and masses*				
				x	Target Organs*				

* Recommended for subchronic rodent studies based on OECD 407 and EPA Guideline 870.3100
+ Organ weights required.

II. RESULTS

A. Observations:

1. Clinical signs of toxicity - There were no clinical signs of toxicity observed.

2. Mortality -

One female at 150 ppm was found dead on day 28, unrelated to BAS 800 H exposure.

B. Body weight and weight gain: Table 2

The body weight and body-weight gains of males at 1350 and 4050 ppm were adversely affected. Dietary

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exposure to BAS 800 H had no effects on body weights of female mice.

Table 2. Mean body weights and body weight gains (g±SD) of males during 28 days of treatment

	♂ (N = 5/group)					
ppm	0	50	150	450	1350	4050
mg/kg bw/d	0	12.8	36.6	112	335	882
Day 0	23.5±0.8	23.5±0.7	23.5±0.5	23.3±0.7	23.5±0.8	23.1±0.5
Day 7	24.6±0.8	24.2±0.5	24.7±0.6	24.4±0.6	24.1±0.6 (-2.1%)	**22.8±0.5** (-7.3%)**
Day 14	26.0±0.9	25.5±0.6	25.5±0.9	25.0±0.7	**24.6±0.7* (-5.5%)**	**23.1±0.6** (-11.4%)**
Day 21	27.3±1.1	26.4±0.7	26.4±0.6	26.1±0.6	**25.6±0.8** (-5.9%)**	**23.3±0.4** (-14.4%)**
Day 28	28.3±1.4	27.1±1.0	27.0±0.8	26.8±0.6	**26.5±0.7* (-6.4%)**	**24.5±0.4** (-13.2%)**
Change day 0-28	4.8±1.3	3.7±07	3.6±0.7	3.5±0.6*	**3.0±0.5** (-38.3%)**	**1.5±0.5** (-69.6%)**
Data taken from Table 1A, pages 59-62 of Report; * ≤0.05; ** ≤0.01; bold values are considered treatment-related						

C. Food consumption and compound intake

1. Food and water consumption: Table 3

Table 3. Mean food consumption (g/mouse/d ± SD) of males during 28 days of treatment

ppm	0	50	150	450	1350	4050
mg/kg bw/d	0	12.8	36.6	112	335	882
Day 7	5.7±0.7	6.4±0.9	6.3±1.2	5.9±1.5	6.2±1.5	5.2±0.8
Day 14	6.2±0.4	6.2±0.6	6.2±1.3	6.0±1.2	5.8±1.2	4.8±0.6
Day 21	6.3±0.9	6.8±1.5	6.6±1.6	6.5±1.3	6.3±1.3	5.2±0.8
Day 28	6.3±1.1	7.0±0.8	6.2±1.2	7.0±1.2	6.6±1.6	5.2±0.5

Data taken from Table IA, pages 69-72 of Report; * ≤0.05; ** ≤0.01; bold values are considered treatment-related

There were no statistically significant findings on food consumption although males at 4050 ppm consistently consumed less food during the study period. There were no overt deviations in the volume of water consumed among the dose groups of the respective sex.

2. Compound consumption Table 1 for the mean daily test substance intake in mg/kg bw/d.

3. Food efficiency

Food efficiency in males at 150 and 4050 ppm was decreased on several days of the study. Due to the spilling of food by mice in all groups, irrespective of the dose, and the lack of consistent trend, these measured deviations in food efficiency were considered incidental.

D. Ophthalmoscopic examination Ophthalmoscopic examinations were not conducted.

E. Blood analyses

1. Hematology Table 4

In males, significantly lower values for red blood cells (RBC), haemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) were observed in males at 1350 and 4050 ppm. In addition, Hb and Hct values were significantly lower in the male mice at 150 and 450 ppm and MCV and MCH were significantly decreased in the males at 450 ppm. Examination of RBC morphology in males revealed increased anisocytosis, microcytosis, and polychromasia at 4050 ppm. Increased polychromasia was also seen in the erythrocytes of the males at 1350 ppm.

In females, significantly reduced values for Hb, Hct, MCV, MCH, and MCHC were found at 4050 ppm. Increased anisocytosis and polychromasia were measured in erythrocytes of females at 4050 ppm.

2. Clinical chemistry Table 4

Serum enzyme examinations revealed dose-dependent, significant increases in alanine (ALT) and aspartate aminotransferase (AST) activities in males at 150, 450, 1350, and 4050 ppm. Significantly higher alkaline phosphatase (AP) activities were also seen in males at 4050 ppm. In females at 1350 and 4050 ppm alanine aminotransferase activities were significantly increased. No treatment related changes were seen in the other serum enzymes or in the cyanide-insensitive palmitoyl-CoA-oxidation of the liver.

Significantly increased urea and total bilirubin levels were found in males at ≥150 ppm and decreased glucose levels were noted in males at ≥450 ppm.

Table 4. Selected hematological and clinical chemistry values

	♂ (N =/group)						♀ (N =/group)					
ppm	0	50	150	450	1350	4050	0	50	150	450	1350	4050
mg/kg bw/d	0	12.8	36.6	112	335	882	0	17.9	63.4	153	446	1621
RBC, 10^{12}/L	10.95 ±0.34	10.70 ±0.35	9.86* ±0.45	10.43 ±0.89	**9.59** ±0.37	**8.80** ±0.73	10.74 ±0.76	10.27 ±0.19	10.15 ±0.36	9.84 ±0.70	10.45 ±0.25	9.79 ±0.39
Hb, mmol/L	9.5 ±0.3	9.2 ±0.2	8.6** ±0.3	8.7* ±0.6	**7.6** ±0.3	**7.0** ±0.4	9.5 ±0.7	9.2 ±0.2	8.9 ±0.3	8.5 ±0.8	8.6* ±0.3	**7.3** ±0.3
Hct, %	49.5 ±1.5	47.8 ±1.5	43.8** ±1.9	45.6* ±3.5	**39.4** ±1.6	**35.2** ±3.2	48.4 ±3.8	46.8 ±0.8	46.0 ±1.6	44.0 ±3.6	35.2 ±1.1	**38.5** ±1.4
MCV, fL	45.2 ±0.2	44.7 ±0.5	44.5 ±0.7	43.8* ±0.7	**41.0** ±0.2	**40.1** ±0.5	45.0 ±1.6	45.6 ±0.6	45.3 ±0.8	44.7 ±1.0	43.3 ±0.5	**39.3** ±0.4
MCH, fmol	0.87 ±0.01	0.87 ±0.01	0.87 ±0.02	0.83** ±0.01	**0.79** ±0.01	**0.80** ±0.03	0.88 ±0.03	0.89 ±0.01	0.88 ±0.01	0.86 ±0.03	0.83* ±0.01	**0.74** ±0.01
MCHC, mmol/L	19.2 ±0.11	19.4 ±0.27	19.5 ±0.28	19.1 ±0.21	19.3 ±0.21	20.1 ±0.73	19.6 ±0.27	19.6 ±0.31	19.4 ±0.20	19.3 ±0.28	19.1* ±0.28	**18.9** ±0.14
WBC, 10^9/L	6.36 ±1.56	6.89 ±4.71	4.79 ±2.02	4.89 ±1.59	3.79 ±2.89	3.97 ±1.72	4.97 ±3.36	2.64 ±2.60	2.62 ±3.12	3.16 ±1.64	3.73 ±3.28	1.60 ±0.39
AP, µkat/L	1.50 ±0.12	1.54 ±0.43	1.44 ±0.20	1.67 ±0.14	1.67 ±0.24	**2.93** ±0.50	2.92 ±0.46	3.08 ±0.36	3.16 ±0.34	2.70 ±0.63	2.07* ±0.17	2.23** ±0.25
ALT, µkat/L	0.91 ±0.17	0.87 ±0.12	3.71** ±1.05	4.45** ±0.89	4.85** ±1.14	**9.17** ±3.90	1.15 ±0.26	1.07 ±0.21	1.27 ±0.32	1.27 ±0.14	2.21** ±0.84	2.91** ±0.41
AST, µkat/L	4.92 ±0.80	6.21 ±1.26	7.18* ±1.20	7.67* ±1.62	8.61** ±0.79	**13.18** ±3.93	7.52 ±0.46	6.76 ±1.39	7.74 ±1.57	7.31 ±1.96	7.79 ±2.04	8.53 ±1.48
Urea, mmol/L	8.66 ±0.95	9.11 ±2.06	11.25** ±1.65	11.81** ±1.12	11.86** ±0.87	10.82** ±0.57	12.64 ±6.32	10.65 ±1.92	11.81 ±2.37	11.16 ±1.89	9.46 ±1.18	11.72 ±0.92

Data taken from Table 1B. pages 67-78 of Report; * ≤0.05; ** ≤0.01; bold values are considered treatment-related

F. Urinalysis Urinalysis was not conducted.

G. Sacrifice and Pathology

1. Organ weight Table 5

In males, there were statistically significant increases in absolute and relative liver weights at ≥150 ppm and increase in spleen weight at 4050 ppm. All other statistically significant weight changes (kidneys, thymus, and brain) were either secondary to the significant terminal body weight decrease in the high dose group or are of no biological significance.

In females, the only treatment-related findings on organ weights were statistically significant increase of absolute and relative liver weights at 1350 and 4050 ppm.

Table 5. Selected organ weight values (mean±SD)

	♂ (N = 5/group)						♀ (N = 5/group)					
ppm	0	50	150	450	1350	4050	0	50	150	450	1350	4050
mg/kg bw/d	0	12.8	36.6	112	335	882	0	17.9	63.4	153	446	1621
BW, g	24.0 ±1.49	22.8 ±1.00	22.7 ±0.96	22.4 ±0.70	**22.0*** ±0.96	**20.2**** ±0.53	18.1 ±1.06	17.5 ±1.09	17.7 ±1.07	18.6 ±0.46	18.2 ±1.01	17.3 ±0.90
Brain mg	448 ±7	440 ±10	451 ±19	451 ±21	441 ±7	450 ±26	Similar values among groups					
Brain %	1.869 ±0.107	1.929 ±0.074	1.991 ±0.118	2.011 ±0.099	2.009 ±0.074	2.226* ±0.148	Similar values among groups					
Kidneys mg	352 ±34	343 ±28	365 ±22	381 ±33	353 ±22	**299**** ±17	Similar values among groups					
Kidneys %	1.465 ±0.121	1.503 ±0.110	1.608 ±0.108	1.695* ±0.121	1.606 ±0.077	1.476 ±0.084	Similar values among groups					
Liver mg	1124 ±42	1051 ±53	**1307**** ±97	**1245**** ±37	**1327**** ±103	**1370**** ±30	825 ±88	857 ±87	819 ±66	866 ±165	**1155**** ±122	**1113**** ±66
Liver %	4.688 ±0.175	4.608 ±0.205	**5.756**** ±0.351	**5.551**** ±0.224	**6.028**** ±0.281	**6.771**** ±0.245	4.564 ±0.301	4.884 ±0.277	4.621 ±0.155	4.662 ±0.869	**6.345**** ±0.432	**6.421**** ±0.182
Spleen mg	60.4 ±7.1	56.0 ±17.7	54.2 ±10.0	49.4 ±7.8	64.6 ±20.5	79.0 ±8.6	Similar values among groups					
Spleen %	0.253 ±0.044	0.246 ±0.083	0.240 ±0.048	0.220 ±0.033	0.294 ±0.094	**0.390**** ±0.043	Similar values among groups					
Thymus mg	42.0 ±5.4	41.6 ±7.8	37.8 ±5.5	36.6 ±5.7	40.6 ±6.3	43.8 ±4.8	Similar values among groups					
Thymus %	0.175 ±0.017	0.182 ±0.031	0.166 ±0.020	0.163 ±0.026	0.184 ±0.023	**0.216**** ±0.020	Similar values among groups					

Data taken from Table 1C. pages 79-82 of Report; * ≤0.05; ** ≤0.01; bold values are considered treatment-related

2. **Gross pathology** There were no treatment-related gross pathological findings.

3. **Microscopic pathology** Table 6

Substance-induced findings were observed in the liver and spleen. In the liver, changes included centrilobular fatty changes (♂ ≥150 ppm; ♀ ≥450 ppm), minimal lymphoid infiltration (♂ ≥150 ppm; ♀ ≥1350 ppm), and extramedullary hematopoiesis (♂ ≥450 ppm). In the spleen, the only finding was extramedullary hematopoiesis (♂ ≥1350 ppm; ♀ 4050 ppm). A slight increase of apoptotic necrosis of lymphocytes was observed in the thymus of treated males at ≥150, which was regarded as a secondary effect of treatment.

Table 6. Selected microscopic findings (number of animals affected)

			♂ (N = 5/group)						♀ (N = 5/group)					
ppm			0	50	150	450	1350	4050	0	50	150	450	1350	4050
mg/kg bw/d			0	12.8	36.6	112	335	882	0	17.9	63.4	153	446	1621
liver	Lymphoid infiltration	gr 1			4	5	5	5					5	5
	Fatty change, diffuse,	gr 1 gr 2	5	5					2	3	1			
	Fatty change, centrilobular	gr 1 gr 2 gr 3 gr 4 gr 5			2 3	5	4 1	1 4	3	2	3	3 2	5	5
	Extramed hemato	gr 1				5	4	5						
spleen	Marked extramedullary hematopoiesis	gr 1 gr 2 gr 3 gr 4	1 1	1	1		1 3 1	5	1 1		1	1 1	2	4 1
	Iron storage, gr 1 gr 2		3 2	3 1	3 1	3	4 1	5						
thymus	Apoptotic necrosis	gr 1 gr 2 gr 3 gr 4 gr 5	1	1	1 1	1 2	2 1	1 2	1 1		1			2 2

Data taken from Table 1C. pages 85-82 of Report; * ≤0.05; ** ≤0.01; **bold** values are considered treatment-related
extramed hemato = extramedullary hematopoiesis; gr = grade

III. DISCUSSION

A. Authors' conclusions

The oral administration of BAS 800 H in the diet to male and female mice over 4 weeks caused signs of general systemic toxicity such as impaired body weight and body weight gain as well as numerous hematological changes indicative of a sideroblastic anemia. The red blood cells, the liver and spleen were recognized as targets of the test article.

The lowest observed adverse effect level (**LOAEL**) in **males** was 150 ppm (36.6 mg/kg bw/d) based on altered hematologic parameters (decreased Hb, Hct), altered clinical chemistry (increases in ALT, AST, urea and total bilirubin) as well as an increased liver weight with associated structural changes in the liver (centrilobular fatty change). The resulting no observed adverse effect level (**NOAEL**) in males was 50 ppm (12.8 mg/kg bw/d).

The **LOAEL** in **females** was 450 ppm (153.1 mg/kg bw/d) based on centrilobular fatty change in the liver. The resulting **NOAEL** in females was 150 ppm (63.4 mg/kg bw/d).

B. Reviewer's comments

The study was properly conducted and reported. The authors' conclusions are valid.

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